



Growth and source-sink relationship in carrot plants biostimulated with Quitomax[®] and Pectimorf[®]

Crecimiento y relación fuente-demanda en plantas de zanahoria bioestimuladas con Quitomax[®] y Pectimorf[®]

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ABSTRACT: The research was developed with the aim of evaluating the influence of chitosan oligosaccharins and oligogalacturonides on the growth and source-sink relationship of the carrot crop. A randomized block experimental design with three replicates was established to evaluate the foliar application of 150 mg ha⁻¹ of QuitoMax[®] and Pectimorf[®] on plants at 20 and 50 days after planting (DAS). Periodic destructive sampling was carried out to determine, among other agromorphological variables: height, leaf area, accumulation and distribution of dry mass in the plant, from which growth rates and source-sink potentials were calculated throughout the biological cycle. At harvest, crop length, diameter, fleshy root weight and yield were evaluated. A direct effect of both oligosaccharins on photosynthetic activity, production and distribution of photoassimilates from leaves to roots in early stages of the biological cycle was evidenced. The results validated the favorable effect of the biostimulants on plant growth and dry mass production with a significant impact of the PectiMorf[®] product (oligogalacturonide mixture) on the commercial quality of the fleshy root and yield at harvest.

Key words: oligosaccharides, plant physiology, biomass production, yield.

RESUMEN: La investigación se desarrolló con el objetivo de evaluar la influencia de oligosacarinas de quitosano y oligogalacturónidos en el crecimiento y la relación fuente-demanda del cultivo de la zanahoria. Se estableció un diseño experimental de bloques al azar con tres réplicas, para valorar la aplicación foliar de 150 mg ha⁻¹ de QuitoMax[®] y Pectimorf[®] en las plantas a los 20 y 50 días de sembradas (DDS). Se realizaron muestreos destructivos periódicos para determinar, entre otras variables agromorfológicas: altura, superficie foliar, acumulación y distribución de masa seca en la planta, a partir de las cuales se calcularon los índices de crecimiento y potenciales fuente-demanda a lo largo del ciclo biológico. En la cosecha se evaluó el largo, el diámetro, el peso de la raíz carnosa y el rendimiento del cultivo. Se evidenció un efecto directo de ambas oligosacarinas en la actividad fotosintética, la producción y la distribución de fotoasimilados, desde las hojas hacia las raíces en etapas tempranas del ciclo biológico. Los resultados validaron el efecto favorable de los bioestimulantes en el crecimiento y la producción de masa seca en las plantas con un impacto significativo del producto PectiMorf[®] (mezcla de oligogalacturónidos) en la calidad comercial de la raíz carnosa y el rendimiento en la cosecha.

Palabras clave: oligosacáridos, fisiología vegetal, producción de biomasa, rendimiento.

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INTRODUCTION

Carrot (*Daucus carota* L.) is the most widespread commercial species of the Umbelliferae family and one of the most widely grown root vegetable crops worldwide, with high economic values (1). In the last 30 years, the growth rate of its consumption has exceeded the population growth rate on a global scale. China is the main producer country with about 16 800 000 tons, followed by Russia (1 565 032 t), the United States (1 346 080 t), Uzbekistan (1 300 000 t) and Ukraine (915 900 t), most of which is marketed in fresh form (2). The world average yield is 22.4 t ha⁻¹, with countries such as Holland, Spain, England and USA standing out with values between 40 and 50 t ha⁻¹ (3).

In Cuba, yields reach 30 to 40 t ha⁻¹, depending on the variety and production conditions; it is one of the most sinked vegetables at any time of the year, due to its excellent taste qualities, the possibility of being consumed fresh or canned, and its relative contribution in vitamins and minerals (4).

Its crop in the country extends, fundamentally, to organoponic conditions and intensive orchards under sustainable production technologies (minimum use of agrochemicals), dependent on high volumes of organic matter not always available, which limits the fertility of the substrates and the biological efficiency of plants. For this reason, the use of biostimulants and biofertilizers is implemented to allow the crop to overcome stress situations in adverse environmental conditions and maximize its intrinsic productive potential to increase yields (5).

In this sense, the biostimulants QuitoMax[®] (a mixture of chitosan polymers) and PectiMorf[®] (a mixture of pectic oligosaccharides or oligogalacturonides), developed by the National Institute of Agricultural Sciences (INCA), have proven to be a promising alternative to positively induce the growth and productivity of several crops, since they exert a proven influence on vegetative and root growth, shorten and enhance the flowering and fruiting period and increase yields through various application forms (6-10).

The present work aims to evaluate QuitoMax[®] and Pectimorf[®] biostimulant effect on growth and the source-sink relationship of carrot crops.

MATERIALS AND METHODS

The research was carried out in the Organoponic of the University of Matanzas (23° 01' 57" N and -81° 30' 31"

W, Lambert Conformal Conical Projection with origin Cuba North), under intensive orchard conditions, during February to May 2017. The carrot variety "New Kuroda" with a biological cycle of 90 to 100 days was used.

An area of 180 m² was selected for the experiment, with typical red Ferrallitic soil (11). A randomized block design with three treatments and three replicates was established, consisting of 20 m² plots (nine experimental plots). A planting frame of 0.10 m between plants and 0.15 m between rows (four rows per bed) was taken into account. The following treatments were evaluated:

- T1: control, no product application.
- T2: foliar application of 150 mg ha⁻¹ of QuitoMax[®] at 20 and 50 DAS.
- T3: foliar application of 150 mg ha⁻¹ of Pectimorf[®] at 20 and 50 DAS.

The doses were selected taking into account the favorable results obtained in other crops with the use of these biostimulants (12,13).

The QuitoMax[®] product was characterized by presenting chitosan polymers with an average molecular mass of 1.35 x 10⁵ g mol⁻¹, a degree of N-acetylation of 12 % and a concentration of 4 g L⁻¹, while PectiMorf[®] presented a composition between 55 and 61 % of galacturonid acid and an active ingredient concentration of 0.75 g L⁻¹.

The application was carried out with a 16 liter Matabi knapsack, wetting all plants homogeneously, at a rate of 0.1 µL plant⁻¹ for QuitoMax[®] and 0.5 µL plant⁻¹ for PectiMorf[®].

Agrotechnical management was carried out taking into account the Technical Guide for the Production of Carrot Crops (4), modified according to the possibilities of the experimental area and the crop development, without applying fertilizers and phytosanitary products. The water needs of the crop were covered with the use of a microjet irrigation system.

The evaluations were carried out at different times of the crop cycle (30, 50, 70 and 90 DAS) by destructive sampling, determining each time, in 10 plants per plot (30 per treatment): height (cm), leaf area (dm²) from the number of active leaves with the use of ImageJ software ver. 1.51 software (14) and the proposed methodologies (15,16); total dry mass (g) and by organs (leaves and stem-root complex). Growth rates were calculated using the expressions shown in Table 1 (17).

Table 1. Formulas and units for determining growth rates in vegetables.

Growth index	Symbols	Formulas	Units
Leaf Area Index	LAI	$\frac{LA_2 - LA_1}{SA}$	Dimensionless
Net Assimilation Rate	NAR	$\frac{W_2 - W_1}{T_2 - T_1} * \frac{NLLA_2 - NLLA_1}{LA_2 - LA_1}$	g dm ⁻² day ⁻¹
Crop Growth Rate	CGR	$\frac{1}{AS} * \frac{W_2 - W_1}{T_2 - T_1}$	g m ⁻² day ⁻¹

Symbols Used: W=Total Dry Mass; T=Time; LA=Leaf Area; SA=Soil Area; NL= Natural Logarithm

From the values of dry mass per organ, the distribution curve of assimilates during the crop cycle and the strength or power of source and plant sink were determined (18). The data were analyzed in terms of plant:

$$\text{Source power} = \text{Source size (Leaf area)} * \text{Source activity (NAR)}$$

$$\text{Sink power} = \text{Sink size (Root dry matter)} * \text{Sink activity (TRC Root)}$$

At harvest, fleshy root length and diameter (cm), as well as fresh mass (g) were evaluated and crop agricultural yield (t ha^{-1}) was estimated.

Data were processed using Statgraphics Plus v.5.1 (19), using a simple rank ANOVA and means were compared by Duncan's Multiple Range test at a $p \leq 0.05$; in the analysis of dynamics, the standard error of the means was estimated.

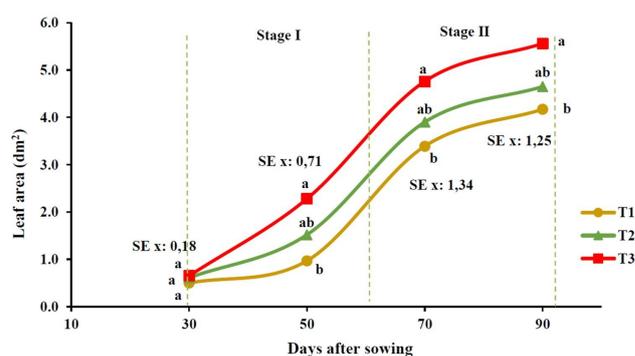
RESULTS AND DISCUSSION

Plant height did not show significant differences between treatments during the phenological crop development (Table 2). However, an increase in this variable was observed in plants treated with biostimulants with respect to the control, starting at 50 DAS.

Quitomax® and Pectimorf® stimulating effect on plant growth has been validated by many authors (20-24). This effect is attributed to the fact that oligosaccharins function as hormonal chemical messengers that regulate growth mechanisms and differentiation in different crops, accelerating the growth process of plants (25). In addition, it is referred that they can stimulate photosynthetic activity; therefore, there is a greater gain of carbon skeletons that are used for the synthesis of new compounds, such as proteins (26).

Figure 1 shows the efficiency of bioproducts in the development of crop leaf area. The best results were achieved in treated plants as a consequence of an increase in leaf size, the oligogalacturonide mixture caused the greatest increase with 25 % above the control, but with no difference with chitosan, the latter did not differ statistically from the control.

It is characteristic in carrot plants to observe a slow growth of foliage in the first stage of vegetative development (30-60 DAS), which then increases steadily in number of leaves between 61 and 97 DAS (Stage II); then the growth



T1: control without application, T2: foliar spray of 150 mg ha^{-1} of Quitomax® at 20 and 50 DAS, T3: foliar spray of 150 mg ha^{-1} of Pectimorf® at 20 and 50 DAS. Different letters in the vertical indicate significant differences according to the Duncan test for $p \leq 0.05$ in the analysis of each sample

Figure 1. Dynamics of leaf area (dm^2) of the carrot plant during the growing cycle.

of foliage slows down (reproductive development phase) and towards the end of the biological cycle of the crop the foliar area tends to stabilize (27). However, the application of biostimulants achieved a higher increase in plant leaf area growth in the first stage, compared to the second, of 26 and 13 % for the oligogalacturonide mixture and chitosan, respectively.

The foliar development seen with Pectimorf® application can be attributed to the fact that this product was able to stimulate the appropriate endogenous hormonal balance, to activate the increase of cell division in buds that originate leaves and promote the synthesis of important substances that act in these processes. Although the mechanisms that can explain in depth the influence of oligogalacturonides on cell division in higher plants have not been defined (28), it has been observed that they are capable of triggering a series of stimuli that accelerate metabolism and enzymatic activity in the cells, which enhances plant growth and development (29).

It can also be related to the increase of photosynthetic efficiency of plant, since it has been observed that the application of this biostimulant increases the content of photosynthetic pigments (chlorophyll a, b and total) in the leaves (26,30,31), which allows a greater capture of photosynthetically active radiation and, therefore, an increase in photosynthetic activity. Other studies indicate that Pectimorf® can enhance photosynthetic capacity in plants due to its effect on the modification of stomatal development patterns (25).

Table 2. Carrot plant height (cm) in the samplings carried out.

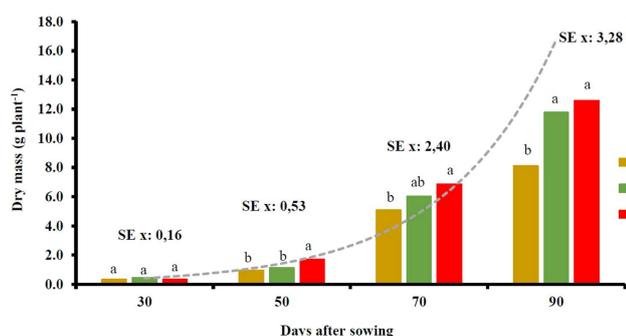
Treatments*	Height plant (cm)			
	30 DAS	50 DAS	70 DAS	90 DAS
T1	23,5 ± 2,66	27,86 ± 2,68	38,89 ± 7,06	46,83 ± 5,36
T2	24,27 ± 1,54	29,91 ± 3,71	42,06 ± 7,45	51,22 ± 5,09
T3	25,33 ± 2,82	29,2 ± 4,97	45,06 ± 8,38	50,0 ± 8,02

* T1: control without application, T2: foliar spraying of 150 mg ha^{-1} of Quitomax® at 20 and 50 DAS, T3: foliar spraying of 150 mg ha^{-1} of Pectimorf® at 20 and 50 DAS ± Standard Error

It is reported that the incorporation of oligogalacturonide mixture in *in vitro* culture media and its foliar spraying in the acclimatization phase increased the number of leaves in banana seedlings (*Musa* spp., AAAB) cultivars FHIA 18 (8) and FHIA 21 (32), as well as the leaf area in seedlings of papaya (*Carica papaya* L.) cultivar Maradol Roja (33) and pineapple (*Ananas comosus* var. *comosus*) hybrid MD-2 (34).

The second application of products accelerated the rate of foliage growth, which had a marked influence on the production of assimilates in plants. The increase in plant biomass is realized from the expanded leaf area as a result of a greater net photosynthetic activity, which is a critical variable for productivity (34).

The increase in dry mass was similar at 30 DAS in all treatments (Figure 2). Statistically significant differences were found after 50 DAS; at the end of the crop cycle, the treatments with both biostimulants showed the highest productivity (11.76 and 12.58 g plant⁻¹, respectively) with increases of 45 and 55 % with respect to the control (8.1 g plant⁻¹), which at all times evaluated presented the lowest absolute values.



T1: control without application, T2: foliar spray of 150 mg ha⁻¹ of QuitoMax[®] at 20 and 50 DAS, T3: foliar spray of 150 mg ha⁻¹ of PectiMorf[®] at 20 and 50 DAS. Different letters indicate significant differences according to Duncan test for $p \leq 0.05$ in the analysis of each sampling

Figure 2. Total dry mass accumulation (leaves and stem-root complex) (g) in carrot plants at different times of the crop cycle.

Oligosaccharins favor plant photosynthetic activity, causing greater biomass accumulation in both aerial organs and roots. Previous authors have shown that foliar PectiMorf[®] application causes increases in the aerial and root mass of radish (35), as well as greater productivity of accumulated biomass in potato tubers (36) and zucchini fruits (*Cucurbita pepo* L.) var. 'Grey Zucchini' (37). Also, different forms of treatment with QuitoMax[®] have increased the formation and enlargement of stolons in potato (22), the production of fresh and dry mass in cucumber fruits (38) and peppers (39), as well as the aerial part and roots in tobacco plants (24).

The determination of growth indices (Figure 3) allowed a better understanding of the growth process and physiological efficiency of the carrot crop. No significant differences were found in the LAI and NAR indicators in plants treated with the

biostimulators, although they did show a better performance with respect to the non-applied control.

Crop growth rate increased as the crop cycle progressed and the formation of the fleshy root began; it was the only one of the indices studied that showed statistically significant differences. The highest values coincided with the moment when there was an increase in the sink potential (70 DAS for the control and between 70-90 DAS with biostimulant application). PectiMorf[®] application caused the highest values, followed by the QuitoMax[®] treatment, which in the first sampling did not differ from the control. At the end of the crop cycle, the biostimulated plants recorded a maximum value of 19.0 g m⁻² day⁻¹, while the untreated plants only reached increases of 10.85 g m⁻² day⁻¹ which shows an increase of 75 % with the use of the products.

The results support the hypothesis that biostimulants favor plant photosynthetic activity (40), although it cannot be ruled out that, in addition, these products facilitate the uptake of nutrients from the soil, something that has been previously demonstrated (41). Thus, PectiMorf[®], due to its potential as a rooting agent (33-42), can favor the formation of roots that enable an efficient supply of water and mineral salts (34) and, therefore, greater plant development.

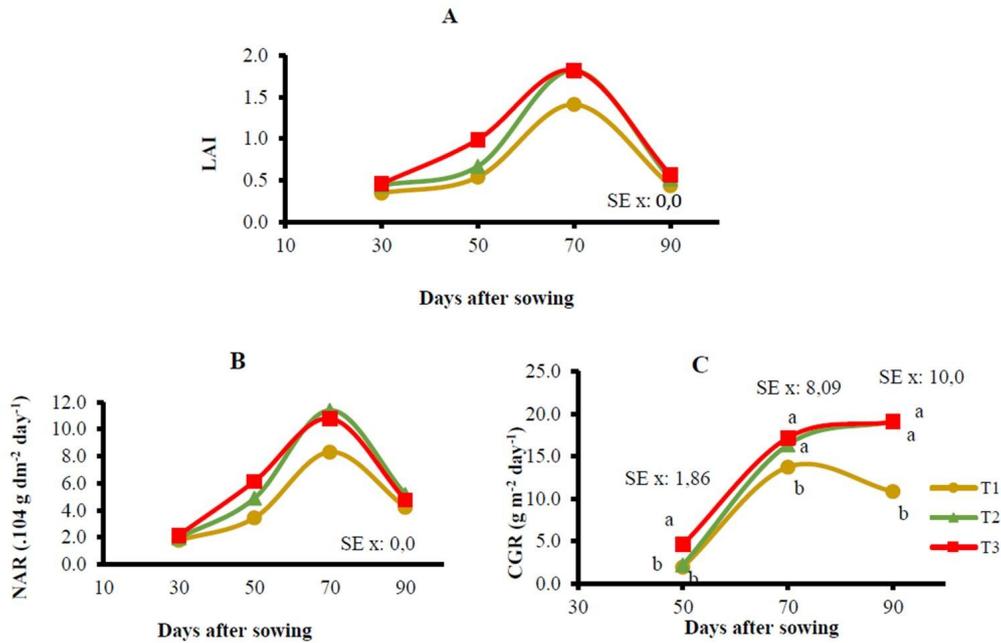
Differences were also found in the treatments evaluated in terms of the maximum points of dry mass accumulation in the plant organs (Figure 4).

The control treatment showed normal behavior in the distribution of dry biomass. A higher percentage of accumulation was observed in the leaves, at 30 DAS, with 61.4 % of the total. Dry mass accumulation in the root was a slow process up to that time, but then began to increase steadily until harvest, where 73.7 % was reached.

To achieve rapid initial growth of young carrot plants, a substantial increase in leaf area in the vegetative phase is important because, due to the morphology of leaves, which have long petioles and segmented blades, much of the incident solar radiation is not intercepted. Therefore, in this phase, a large part of assimilates must be destined to the formation of leaves (43). When the foliar system reaches an appropriate development, the partitioning of assimilates to the foliage decreases, with the consequent increase in their mobilization to the root (44). With the onset of thickening, roots become the main sink.

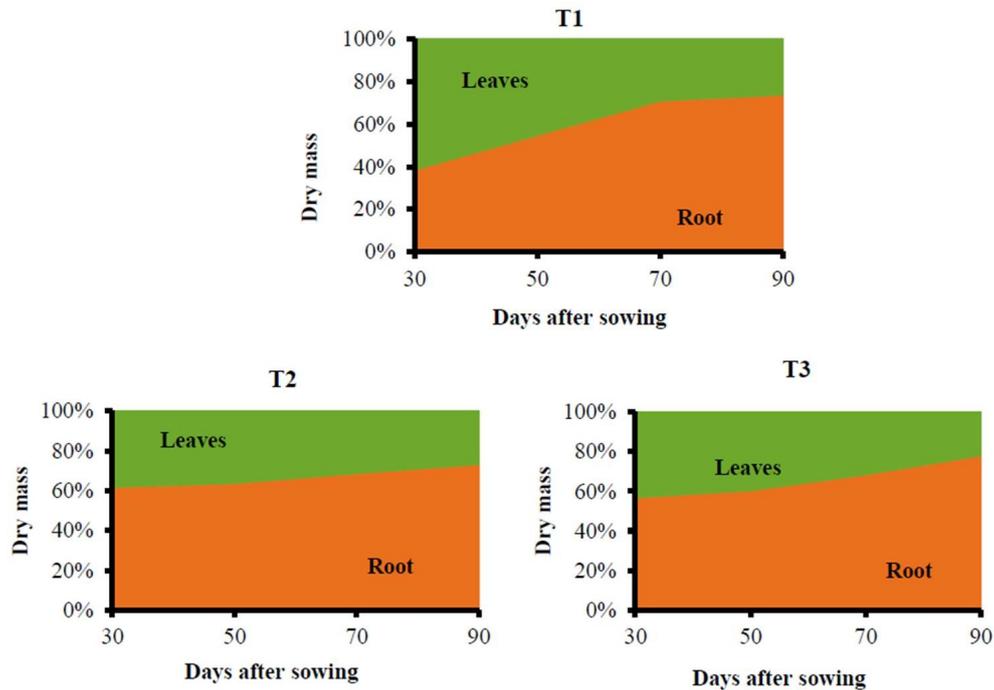
Plants treated with biostimulants showed a greater accumulation of assimilates in the root (61.8 and 56.9 % for QuitoMax[®] and PectiMorf[®] applications, respectively) than in the leaves (38.2 and 43.1 %) from the first sampling.

The partitioning of assimilates between the aerial part and the root, during the carrot crop cycle, is an expression of the interaction between genetic, environmental and cultural factors (45). The observed behavior can be attributed to the fact that the foliar application of bioproducts increased levels of traditional hormones such as gibberellins and abscisic acid (ABA), substances that are closely related to the process of dry mass distribution in plants and the tuberization induction (46).



T1: control without application, T2: foliar spray of 150 mg ha⁻¹ of QuitoMax® at 20 and 50 DAS, T3: foliar spray of 150 mg ha⁻¹ of PectiMorf® at 20 and 50 DAS. Different letters in the vertical indicate significant differences according to the Duncan test for p<0.05 in the analysis of each sample.

Figure 3. Carrot crop growth indices: Leaf Area Index (A), Net Assimilation Rate (B), Crop Growth Rate (C).

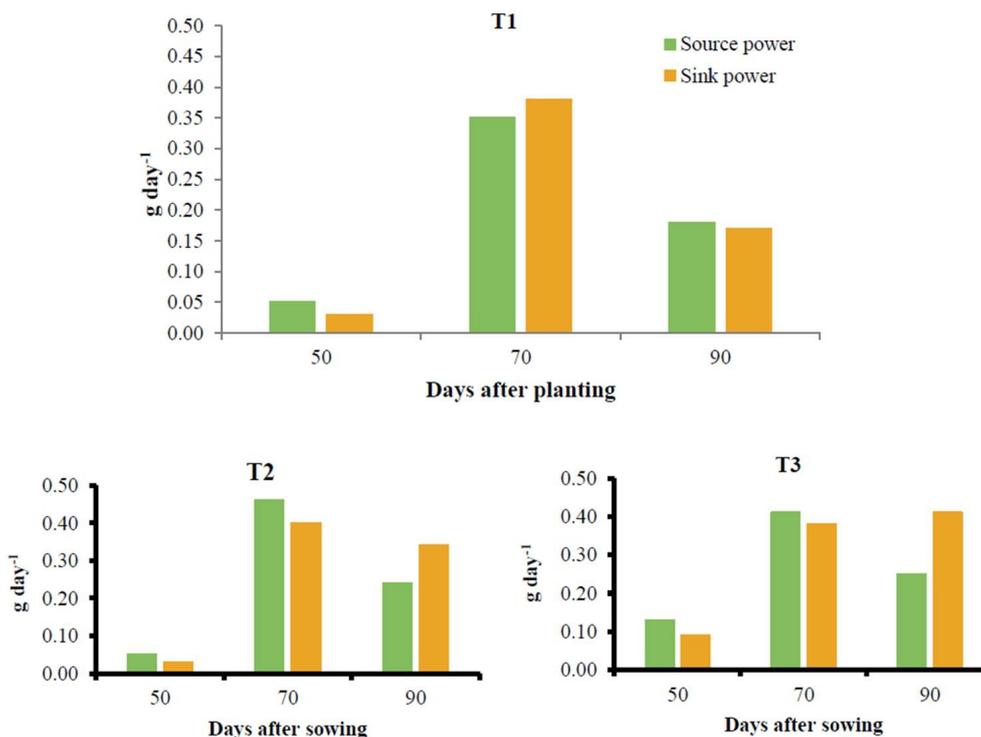


T1: control without application, T2: foliar spray of 150 mg ha⁻¹ of QuitoMax® at 20 and 50 DAS, T3: foliar spray of 150 mg ha⁻¹ of PectiMorf® at 20 and 50 DAS.

Figure 4. Dynamics of dry mass percentage distribution by organs (leaves and root) with respect to the total in carrot plants.

Figure 5 shows differences in the behavior of the source-sink potentials of plants in the evaluated treatments. In the control, the maximum potential of leaves as source tissue was obtained at 70 DAS, with a value of 0.35 g day⁻¹; the same behavior was presented at this time by the root sink potential

with a value of 0.38 g day⁻¹. This period corresponded to the moment of greatest translocation of assimilates from leaves to roots. At the end of the cycle for this treatment, both potentials decreased as a result of the decrease in the LAI and the rate of root weight gain near harvest.



T1: control without application, T2: foliar spray of 150 mg ha⁻¹ of QuitoMax[®] at 20 and 50 DAS, T3: foliar spray of 150 mg ha⁻¹ of PectiMorf[®] at 20 and 50 DAS, T4: foliar spray of 150 mg ha⁻¹ of PectiMorf[®] at 20 and 50 DAS

Figure 5. Source-sink potentials in carrot plants.

Plants that received QuitoMax[®] applications reached values above 70 DAS, with maximum source and sink potentials for leaves and roots of 0.46 and 0.40 g day⁻¹, respectively; towards the end of the crop development cycle the source potency decreased markedly, while the sink strength maintained high values (0.34 g day⁻¹) indicating that the root continued to accumulate reserves until harvest time, increasing crop productivity.

On the other hand, the application of PectiMorf[®] caused a higher source power in plants at 70 DAS (0.41 g day⁻¹) and a higher root sink potential at 90 DAS (0.41 g day⁻¹). In the latter sampling, a considerable increase in sink power was observed, which represents, that the highest translocation and discharge of photoassimilates in the root took place in this period.

Dry mass assimilation and its distribution within the plant are important processes that determine crop productivity (47). The production of assimilates by the leaves and the point to which they can be accumulated by the sink, represented in

this case by the harvested root, defines yields (48). Higher values of the potentials between source and sink organs and in the net assimilation rate (NAR) can translate into a greater biomass accumulation at times close to physiological maturity, reaching higher yields (49).

Table 3 shows results on yield and the components that determine it at the time of harvest. The length of the fleshy root did not differ between treatments, but the diameter, the mass of the fleshy root and, therefore, the yield were higher in plants treated with PectiMorf[®] and differed significantly from the control and chitosan application.

Biostimulants had a positive effect on the agricultural yield of carrot, proving to be effective in the productivity of treated plants, taking into account the increases with respect to the control of 22 % with QuitoMax[®] (although without differences with the control) and 56 % with PectiMorf[®]. Results achieved are superior to those reported for this same variety with the use of biofertilizers based on efficient microorganisms (50).

Table 3. Yield and its components in carrot plants.

Treatments [*]	Fleshy root length (cm)	Fleshy root diameter (cm)	Fleshy root mass (g)	Yield (t ha ⁻¹)
T1	10,14 ± 2,80	2,41 ± 0,36 b	47,85 ± 8,05 b	31,5 ± 2,10 b
T2	11,22 ± 2,53	2,56 ± 0,44 b	58,60 ± 7,63 b	38,70 ± 1,25 b
T3	11,39 ± 1,39	3,33 ± 0,25 a	74,41 ± 17,46 a	49,11 ± 3,26 a

* T1: Control without application, T2: Foliar spray of 150 mg ha⁻¹ of QuitoMax[®] at 20 and 50 DAS, T3: Foliar spray of 150 mg ha⁻¹ of PectiMorf[®] at 20 and 50 DAS. Different letters in the vertical indicate significant differences according to the Duncan test for p ≤ 0.05 in the analysis of each sample. ± Standard Error

In the literature, there are no antecedents of the use of oligosaccharins in carrot cultivation, but it has been demonstrated that applications of PectiMorf® increase yields in radish (35), garlic (7) and potato (9,21,36), as well as the quality of the tubers obtained. The positive effect of the oligogalacturonide mixture on the yield of other non-tuberous crop species such as beans (13), pumpkin (37) and papaya (51) has also been corroborated. Oligosaccharins (and products containing them) have been reported as important yield biostimulatory macromolecules in several crops, with increases ranging from 10 to 60 % above controls, depending on the application rates experimented, the crop and the locations and soils treated (52-54).

CONCLUSIONS

- The carrot crop responded favorably to the foliar application of biostimulants, with a significant impact of the product PectiMorf® (T3) on the increase in leaf area and dry mass production. The differences found in the crop growth rate (CGR), the higher increases in the net assimilation rate (NAR) and the differences in the behavior of the source-sink potentials of plants, with the application of the biostimulants, show their direct effect on the photosynthetic activity, the production and distribution of photoassimilates from leaves to roots in early stages of the biological cycle.
- The foliar application of 150 mg ha⁻¹ of PectiMorf® at 30 and 50 days after planting showed a better productive response of the plants and the commercial quality of the fleshy root, expressed in a notable increase in yields.

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